Nano-fabricated Size Exclusion Chromatograph

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ABSTRACT

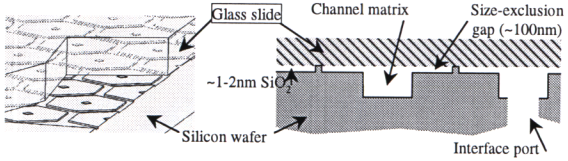
This poster describes the development of a nano-fabricated size exclusion chromatograph (nSEC) based on the principle that molecules traveling through a microcolumn containing nano-fabricated features will have characteristic elution times that directly correlate to molecular weight.

INTRODUCTION

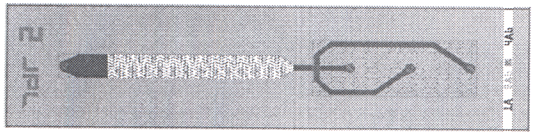
In size exclusion chromatography, a subset of high-pressure liquid chromatography, molecules are separated based on their retention time in a column consisting of small (~10μm) closely packed silica or polymer beads with uniform nanopores ranging from 10 to 1000nm [1]. The molecular diameter determines the analyte's retention time in the column as the ratio of time spent in the nanopores increases with decreasing molecular size. Thus molecules elute in order of decreasing size and can be detected with a variety of methods. The resulting chromatogram yields molecular identification based on peak elution times and molecular concentrations that can be inferred from the peak size.

DEVICE DESCRIPTION

The nSEC's nano-fabricated features, analogous to the traditional SEC's bead nanopores, consist of size-exclusion gaps defined in the z-direction over a matrix of microchannels in the x-y plane, similar to the interstices between the beads (Fig. 1A). A sample plug is introduced into the column via an on-chip injection cross (Fig 1B).



(A)



(B)

Figure 1: (A) 3-D and cross-sectional views of nSEC schematic (B) Top view of nSEC device

DEVICE FABRICATION

Ion milling is used to define the 100nm tall support posts in silicon, followed by reactive ion etching of the channel matrix (3μm wide x 0.4μm deep) (Fig. 2). A ~1-2nm thermal oxide layer is grown over the entire wafer to create a chemically uniform surface. Interface ports are water-jet drilled and finally, a co-efficient of thermal expansion matching cover glass is anodically bonded to the micromachined wafer forming the device roof.

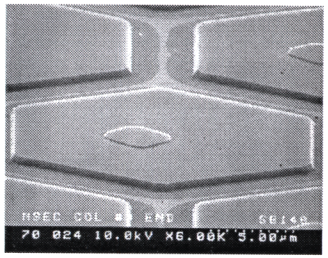


Figure 2: nSEC channel matrix and 100nm tall support posts

THEORETICAL MODEL

We have developed a geometric model for nSEC separations of nanospheres based on diffusion of the spheres between the eluent flow in the microchannel matrix and that in the size exclusion gap (negligible). The diffusion distribution constant, K_i, describes each sphere's probability of entering the size exclusion gap. The constant for planar slab pores is used [2];

$$K_i = 1 - \frac{r_i}{a}$$

where r_i is the molecular radius and a is half the planar gap.

Differences in the diffusion distribution constant for the different sizes beads determines separation. As the spheres pass through the nSEC, the concentration of spheres in the mobile phase, c_m, decreases due to dilution by the fraction of spheres able to enter the gap, c_s, the concentration of spheres in the stationary phase:

$$c_m = \frac{V_m c_i}{V_s K_i + V_m} \qquad c_s = K_i c_m$$

where V_m is the mobile phase volume, V_s is the stationary phase volume, and c_i is the initial molecule concentration.

Diffusion between the mobile and stationary phases as the eluent pushes the spheres through the column, broadens the initial length, ΔL_{ini}, of the sample plug. Assuming a triangular peak at the column exit, the exit band length in terms of time, Δt, is determined by mass balance,

$$\frac{c_{\max}}{2}\left(a_m u \Delta t\right) = \frac{c_s V_s \Delta L_{ini}}{L_c}, \quad c_{\max} = c_s$$

where c_{max} is the maximum concentration of the peak equal to the analyte concentration in the stationary phase, a_m is the cross-sectional area of the matrix channels, u is the mobile phase velocity, and L_c is the length of the column. Retention time, t_r, at which a band of spheres exits the column, is inversely proportional to the band's velocity, which can be determined by the mass fraction, m_f, the ratio of analyte mass in the stationary phase to the analyte mass in the mobile phase [2]:

$$m_f = \frac{c_s V_s}{c_m V_m}, \quad t_{r,i} = \frac{L_c}{\left(\frac{u}{1+m_f}\right)}$$

A normal Gaussian distribution of the sphere concentrations in the stationary phase, $c_{s,i}$, at $t_{r,i}$ over Δt approximates the chromatogram. Assuming given sphere diameters and initial concentrations, a size exclusion gap of 100nm, a sample volume of 0.036pL, a flow rate of 1.5nL/min (@55psi), and negligible surface interactions, our model yields the chromatogram in Figure 3.

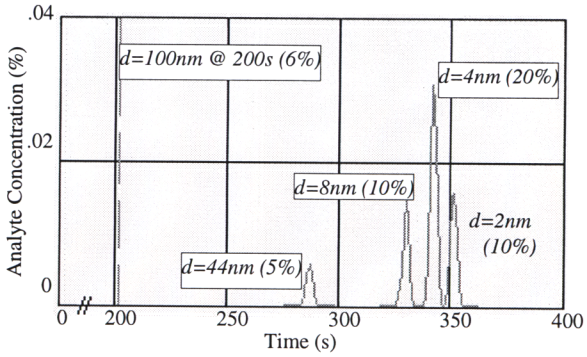


Figure 3: Chromatogram of modeled nSEC separation

CONCLUSIONS

Development of the nSEC will continue with experimental separations of flouresently labeled nanobeads. A nanofluidic injection system consisting of a coupled-syringe pump driven by DC-servo/piezo stack actuators will control fluid flow at ultra low rates. With the ability to precise control nano-size features in a chemically uniform, easily modified SiO_2 surface, the nSEC may offer highly sensitive separations of a variety of analytes.

REFERENCES

1) Skoog, D, Holler, F.J., Nieman, T., Principles of Instrumental Analysis 5th Ed. Hardcourt Brace College Publishers, Philadelphia, USA, 1998, pp. 726.
2) Yau, W.W., Bly, D.D., Kirkland, J., Modern Size-Exclusion Liquid Chromatography: Practice of Gel Permeation and Gel Filtration Chromatography, John Wiley and Sons, New York, USA, 1979.

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